A COMPARATIVE STUDY OF EFFICACY OF LETROZOLE VERSUS CLOMIPHENE CITRATE IN OVULATION INDUCTION IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME

Dissertation submitted to

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CERTIFICATE

This is to certify that the dissertation titled **COMPARATIVE STUDY OF EFFICACY OF LETROZOLE VERSUS CLOMIPHENE CITRATE IN OVULATION INDUCTION** submitted by **Dr. G. THIRIPURASUNDARI** to the faculty of Obstetrics and Gynaecology, The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D. Degree (Obstetrics and Gynaecology) is a bonafide research work carried out by her under our direct supervision and guidance.

Dr. J. MOHANASUNDARAM
M.D. D.N.B., Ph.D.,
Dean,
Madras Medical College,
Chennai – 600 003.

Dr. REVATHY JANAKIRAM
M.D. D.G.O., M.N.A.M.S.
Director,
Institute of Obstetrics and Gynaecology
Madras Medical College,
Chennai – 600 003.

DECLARATION

I hereby declare that the study entitled **COMPARATIVE STUDY OF EFFICACY OF LETROZOLE VERSUS CLOMIPHENE CITRATE IN OVULATION INDUCTION** was done by me in the Institute of Obstetrics and Gynaecology (IOG), Madras Medical College, Chennai – 600 003, during the period of my PG study for MD Branch II Obstetrics and Gynaecology from 2007-2010.

This Dissertation to Dr. M.G.R. Medical University is in partial fulfillment of university regulations for the award of MD Degree in Obstetrics and Gynaecology.

Dr. G.Thiripurasundari
M.D. P.G. (Obstetrics and Gynaecology)
Institute of Obstetrics and Gynaecology
Madras Medical College,
Chennai - 600 003.

Place :

Date :

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CONTENTS

PHYSIOLOGICAL BASIS OF OVULATION

The Ovarian cortex at puberty contains hundreds of thousands of primordial follicles (1). Initially independent of gonadotropins, a cohort (hundreds) of primordial follicles is recruited to grow (2). During the early follicle development, the oocyte enlarge and the granulosa cells proliferate to form a preantral follicle. Over 3 – 6 months, the follicle develops FSH receptors in the granulosa cells and LH receptors in the theca cells (1). At this stage, antral follicles become acutely dependent on FSH for further development (1). Just before menses, falling estrogen levels result in with drawal of negative feed back centrally leading to increased gonadotropin levels (3). FSH stimulates granulosacell proliferation and differentiation, with the development of more FSH receptors and the production of aromatase (4). LH stimulates androstenedione production by theca cells that diffuses in to the granulosa cell providing substrate for estrogen secretion. This step is catalysed by the aromatase enzyme, which is induced by FSH. The so called two – cell, two – gonadotropin theory (5,6) postulated that FSH concentrations must exceed a certain level (FSH) threshold) before follicular development will proceed.

The duration of this period in which the threshold is exceeded (the FSH window) is limited in the normal cycle by a gradual decrease in FSH, occurring in the early mid follicular phase as a response to negative feed back from rising estrogen levels produced by the larger follicles (7). Smaller follicles, with fewer FSH receptors, are no longer stimulated to grow by FSH levels below the FSH threshold and undergo atresia (8). Therefore, generally only one follicle reaches the stage of ovulation each cycle, despite the fact that hundreds of primordial follicles, the number of which varies depending on a woman's age. FSH induces LH receptors

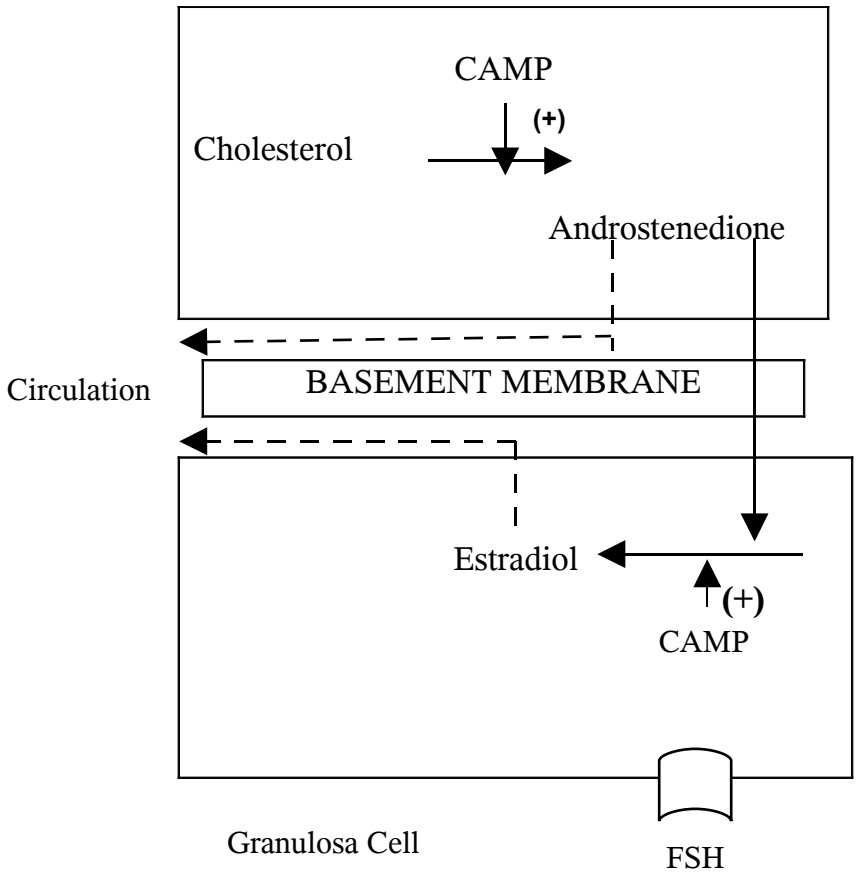
in larger antral follicles above 1 cm in diameter (9). Rapidly increasing levels of estradiol produced by the mature preovulatory follicle precede the mid cycle LH and FSH surge that will initiate ovulation. The circulating level of estradiol determines the time of LH surge. The alteration in steroidogenic pathway results in progesterone as the primary steroid hormone produced after leutinization (10). The corpus luteum retains the ability to produce estrogen. The demonstration of multiple follicular growth (11) suggests that some estrogen in the luteal phase is contributed by growing follicles that will under go atresia (12).

The corpus luteum undergoes regression with a fall in progesterone and estrogen and the onset of menses. FSH levels rise with withdrawal of estrogen negative feed back and the next cohort of follicles begins to develop.

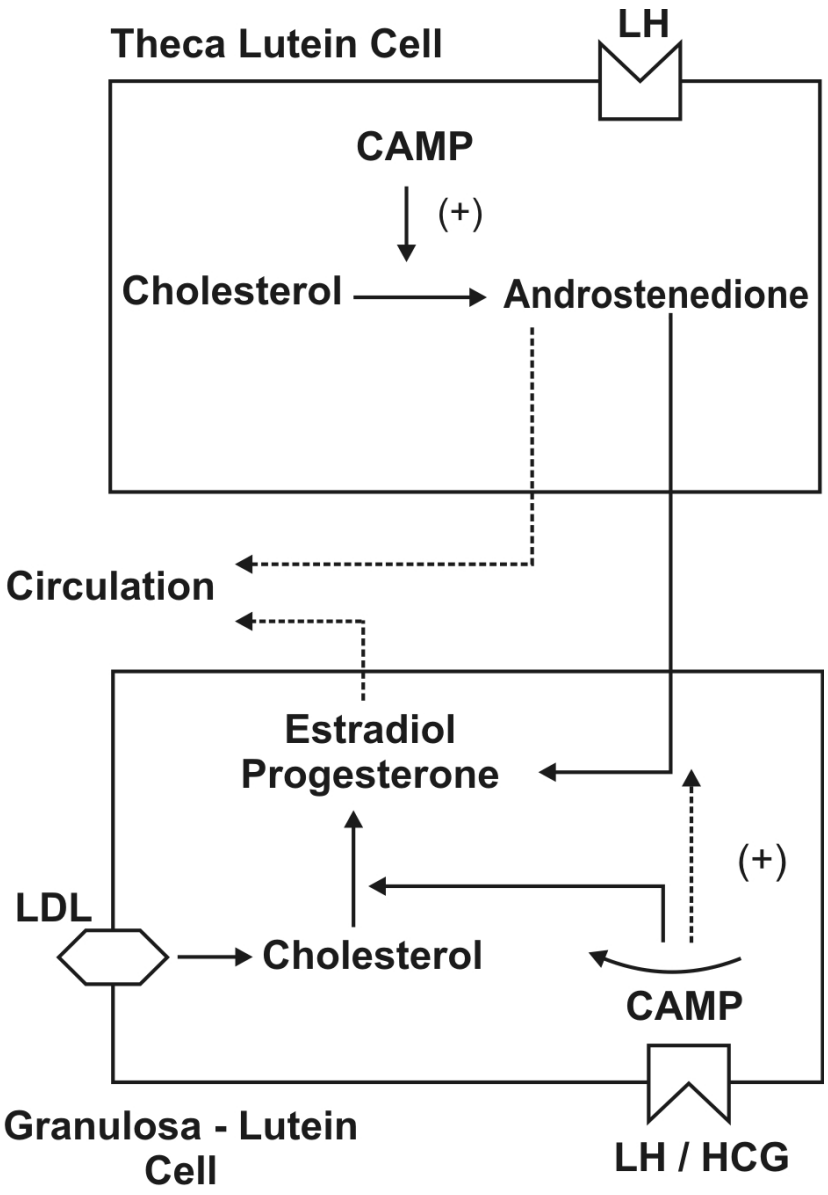
In summary, normal follicular development culminates in ovulation of a nature oocyte, followed by the development of a corpus luteum producing adequate amount of progesterone. This sequence of events is orchestrated by the interaction of local ovarian factors and endocrine factors from the pituitary and hypothalamus. The presence of subtle abnormalities despite the occurrence of ovulation may be responsible for unexplained infertility.

FUNDAMENTAL TENET OF FOLLICULAR DEVELOPMENT
TWO CELL TWO GONADOTROPIN THEORY (13,14,15)

Theca cell


CAMP
(+)
Cholesterol
Androstenedione
Circulation
BASEMENT MEMBRANE
Estradiol
(+)
CAMP
Granulosa Cell
FSH

LUTEAL PHASE



ANOVULATION

Anovulation occurs occasionally in all women. It is probably not uncommon during the first 2 or 3 yrs of menstrual life and after the age of 40 yrs, the incidence may rise to as high as 1 in 5 cycles. In the active reproductive period, however not more than 1 in 50 regular cycles are anovular. For practical purpose, in mature women regular menstruation means regular ovulation.

DIAGNOSIS OF OVULATION

Analysis of symptoms

I. Cyclical Bleeding: Regular normal menstrual loss is strong presumptive evidence of monthly ovulation.

II. Ovulation pain (Mittleschmerz): Some discomfort in the hypogastrium or in one or other iliac fossa for 12 – 24 hrs just before or just after ovulation.

III. Ovulation Bleeding or discharge (Mittelblut) : Slight loss of blood or of mucus tinged with blood at the time of ovulation, sometimes associated with ovulation pain.

IV. Premenstrual Mastalgia: Premenstrual pain and tenderness in the breasts is related to corpusluteum action.

V. Temperature Changes : The biphasic chart is evidence of ovular menstruation. Rise of 0.2 – 0.5° C in the luteal phase is due to thermogenic action of progesterone.

VI. Changes in Endometrium: The evidence that ovulation has occurred predicted by secretory activity in the gland during the week preceding or at the onset of menstruation by endometrial biopsy. But is not accurate.

VII. Change in cervical Mucus: At the time of ovulation mucus is thin and profuse with great elasticity and with stand stretching up to 10cm – spinn barkeit or thread test.

Vaginal Smear cytology	**Basal Cells**	**Intermediate Cells**	**Superficial Cells**
Estrogenic Phase	0	20	80
Progesteronic Phase	0	80	20

IX. Hormonal Assays: Ovulation is confirmed by an estimation of mid luteal plasma progesterone level. A minimum of 6.5ng/ml indicate ovulation. Ovulation occur 10 – 12 hrs after LH peak or 34-36 hours after the initial rise in mid cycle LH.

Hormonal levels in different phases of Menstrual cycle

Hormone	F.P	Ovulation	L.P	Menstrual
FSH	5 – 15 m IU/ml	12 – 30	2 – 9	3 – 15
LH	6 – 14 m IU/ml	25 – 100	2 – 13	3 – 12
E2	100 – 200 pg/ml	300 – 500	100 – 200	
P	1 ng/ml	-	15 ng/ml	

Ultrasound

Helpful in the induction and confirmation of ovulation. The graffian follicle grows at the rate of 1 -2 mm/day. Attains the size of 20 mm or more at ovulation

Evidence of

- Sudden shrinkage in size of a follicle
- Appearance of free fluid in POD
- Regrowth of the collapsed cyst

XI Direct observation: The finding of an active corpus luteum on inspecting the ovary (evidence of stigma) during laparoscopy or laporatomy.

OVARIAN DYSFUNCTION

According to WHO (1976) classification

Group I → Hypothalamic pituitary failure

- Anorexia nervosa
- Kallmann's syndrome
- Sheehan's syndrome

Group II → Hypothalamic pituitary dysfunction

- PCOS
- CAH
- Adrenal tumours
- Androgen producing ovarian tumours

Group III → Ovarian failure

- Turner syndrome
- Pure gonadal dysgenesis
- Swyer syndrome
- Autoimmune disorders

- RT/CT

- Infections

Group IV → Congenital or Acquired genital tract disorders (Eg) Imperforate hymen, MRKH syndrome, Asherman's syndrome.

Group V → Hyperprolactinemia with SOL (Eg) pituitary adenoma.

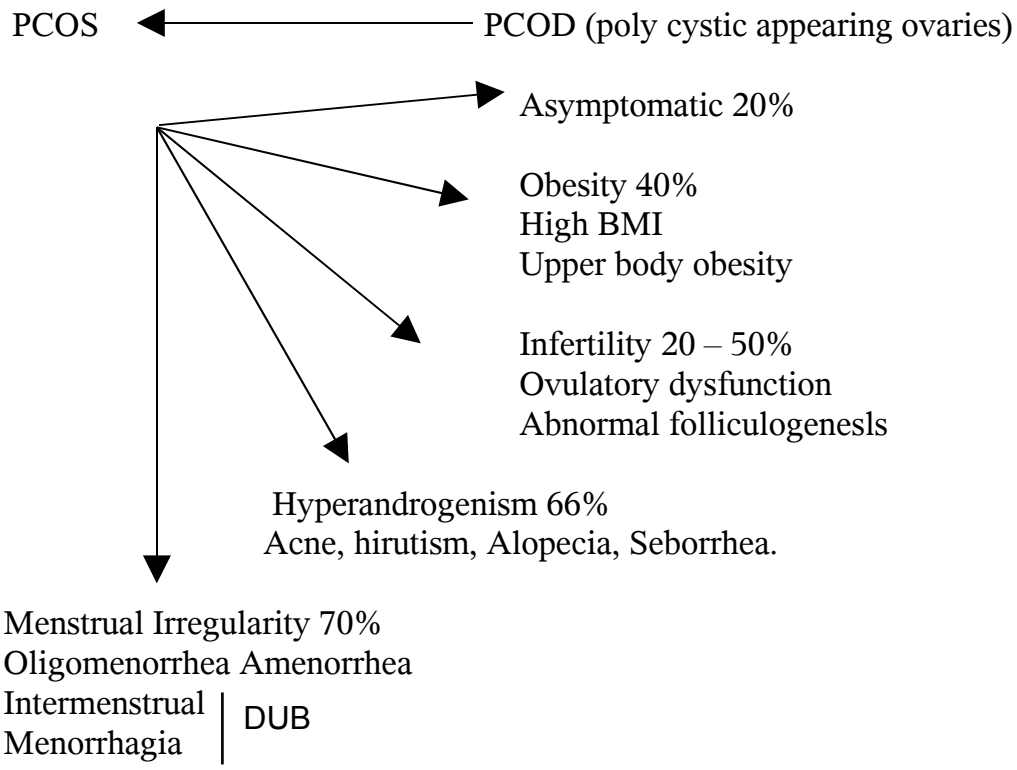
Group VI → Hyperprolactinemia without SOL (Eg) Hypothyroidism, CRF, drug induced.

Group VII → Amenorrhea with an SOL with normal or low PrL (Eg) craniopharyngioma.

POLYCYSTIC OVARIAN SYNDROME (PCOS)

It is a complex disorder with heterogeneous clinical and endocrine factors. Incidence in the reproductive age group is around 20 – 22% (Polson et al 1988) (16). Polycystic ovaries have its origin during adolescence and is thought to be associated with increased weight gain during puberty (Belen and Danger 1995) (17).

Clinical features :



CRITERIA (1) The NICHD (1990)

(2) The ESHRE & ASRM / Rotterdam (2003)

(3) AES criteria (2006)

Rotterdam Criteria (1) Oligo / chronic anovulation

(2) Clinical / Biochemical Evidence of Hyperandrogenism

(3) USG E/o polycystic ovaries exclude other causes of hyperandrogenism

Biochemical / Endocrine Abnormalities

1. Raised LH
2. Raised LH: FSH ratio
3. Raised Androgen – Testosterone and androstenedione
4. Raised circulating estrogen
5. Raised fasting Insulin
6. Raised fasting glucose
7. Raised prolactin
8. Decrease in SHBG
9. Raised testosterone : SHBG ratio

Syndrome X (Dysmetabolic Syndrome) (18) :

1. Dysliplidemia - TGL > 140 mg/dl

   HDL < 40 mg/dl

2. Insulin resistance, fasting plasma Insulin > 110 mg/dl

3. Obesity BMI > 25 kg /m^2 or

   WHR > 0.85 or waist > 100 cm

4. BP > 140 / 90 mm Mg

5. ↑serum uric aid or ↑PAI – 1 levels

OVULATION INDUCTION

Ovulation Induction refers to the therapeutic restoration of the release of one egg per cycle in the woman who either has not been ovulating regularly or has not been ovulating at all. Although it is usually acceptable for OI to result in the release of two eggs, one should avoid the ovulation of more than two eggs in an effort to minimize the risk of OHSS and multiple gestation.

Appropriate patient selection can be a significant determinant of the success of OI among PCOS patients.

Once ovulation has been documented for a particular treatment. Patients should be mentally prepared to continue with that regimen for at least 3 cycles. Because for most treatment the rate of a pregnancy occurring per therapeutic cycle is the same for each of the first three cycles.

It is mandatory to exclude male factor infertility by means of semen analysis and bilateral tubal patency by HSG.

OVULATION INDUCTION DRUGS

Induction Agents

1. SERMS - Clomiphene citrate

2. Aromatase Inhibitors - Letrozole.

Adjuncts

(1) Insulin sensitizers

(2) Gonadotrophins → Menotropins
Gonadotrophins → recombinant Gonadotropins

(3) Gn RH → pulsatile Gn RH
Gn RH → Gn RH agonist → with HMG alone
Gn RH agonist → with FSH / HMG

(4) Dopamine Agonists

(5) Dexamethasone

(6) Pretreatment OCPs.

PHARMACOLOGY OF DRUGS

CLOMIPHENE CITRATE

For more than 40 yrs, CC has been the most commonly used oral agent for OI. In 1961, Greenblatt et al., (19), published the first results of OI achieved by CC (at that time known as MRL / 41). But CC is considered pregnancy risk category X. CC belong to the category of SERMs.

SERMs :

1. Designer estrogens with combined effects of estrogen antagonism and partial agonism.

2. Nonsteroidal analogues with tissue specific activity

3. Differential modulation of estrogen receptors results in multiple target system.

CLASSIFICATION

1. I generation Triphenyethylene compounds :

    - Tamoxifen
    - Toremifene
    - Droloxifene
    - Idoxifene
    - Clomifene

2. II generation Benzothiophene compounds

- Raloxifene

3. Dihydronaphthylenes

- Trioxifene

4. Tetrahydronapthylenes

- Lasofoxifene

5. Benzopyrans - Ormeloxifene

- Levormeloxifene

NEWER SERMs

- Ospemifene
- Arzoxifene
- SP 500263
- CHF 4056
- CHF 4227
- FCI 271a
- LY 1271a
- LY 353381
- GTx 006

Other uses of SERMs

- HRT

- Breast ca – adjuvant
- Prevention of osteoporosis
- Cardio protective agent

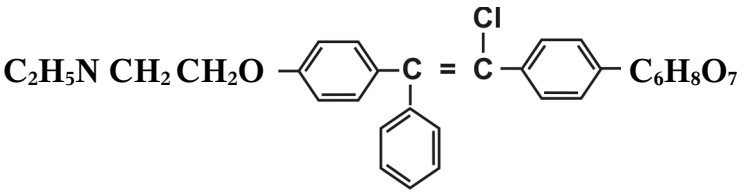
CC is an orally administered nonsteroidal ovulatory stimulant designated chemically as

Molecular formula : 2 –(4 –(2 – Chloro -1, 2- diphenyl ethinyl phenoxy) – N – N – diethyl Ethan amine

$C_{28}\ H_{28}\ C_{1NO}\ .\ C_8\ H_8\ O_7$

Molecular weight - 598.10

Structure



Isomers : CC is a diastereomeric mixture of two geometric isomers

30% Zuclomiphene (cis isomers, formerly called Transclomiphene) (Emst et al., 1976).

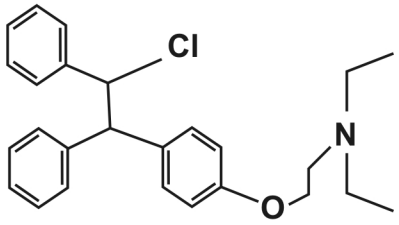
62% enclomiphene (Trans isomer – formerly called is clomiphene) (Holtkamp et al., 1987).

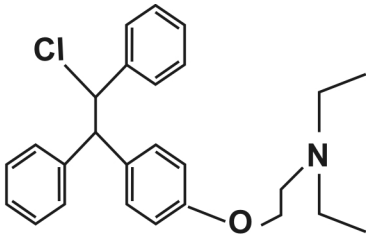
Zuclomiphene is mildly estrogenic as well as antiestrogenic.

Enclomiphene is entirely antiestrogenic.

Zuclomiphene is approximately 5 times as potent as enclomphine in inducing ovulation. (20,

21).





ZUCLOMIPHENE **ENCLOMIPHENE**

Mechanism of Action

- CC acts as competitive antagonist of 17B Estradiol at the level of cytoplasmic nuclear receptor complex.

- Blockade of estrogen receptors in the hypothalamic arcuate nucleus leads to an increase in Gn RH.

- Increases the pituitary sensitivity to Gn RH (23).

- Increases pulse amplitude but not frequency in anovulatory women with PCOS, in whom the Gn RH pulse frequency is already abnormally high (24).

- FSH and LH secretion is increased (Dickey et al., 1965) (25).

- Also have a direct effect on the ovary making the granulosa cells more sensitive to pituitary.

- Hence serum progesterone and estradiol concentration rises during luteal phase in a direct dose response relation ship (Hammond et al., 1982 ; Fukuma et al., 1983). Supraphysiological levels of estrogen can occur without central suppression of FSH because the normal ER-mediated feed back mechanisms are blocked as a result of prolonged ER depletion (22). As a result the FSH window being extended – leading to multiple follicle growth and a higher multiple pregnancy rate with CC.

Anti estrogenic effects of CC

The endometrium is one of the most important target of antiestrogenic effect of CC (26). A reduction in endometrial thickness below the level thought to be needed to sustain implantation was found in up to 30% of women receiving CC (27). Endometrial thickness < 5 -6 mm is associated with failure to conceive (27,28,29). Deleterious effect on EM is demonstrated by reduction in glandular density and increase in vacuolated cells (30).

PHARMACOKINETICS

CC is excreted principally through intestine 5 days after oral administration 51% has been excreted. However some CC continues to be excreted for at least 6 wks (Mikkelson et al., 1986).

- Peak plasma concentrations of zuclomiphene occurs 6 hrs after oral administration of 50 mg.

- A steady state 25% reached at 48 hrs remains constant for the next 14 days.

- Single dose studies showed that Zuclomiphene (cis) has a longer half life than enclomiphene (trans). Detectable level persisted for longer than a month.

INDICATION AND DOSAGE

1. Anovulatory Infertility; PCOS
2. Unexplained Infertility
3. Amenorrhea – Galactorrhea syndrome
4. Psychogenic amenorrhea
5. Post oral contraceptive amenorrhea
6. Certain cases of secondary amenorrhea of undetermined etiology.

DOSAGE

Starting dose 50 mg / day. A baseline TVS performed on D1 to D3 to exclude ovarian

cyst. Therapy is begun with in the first 5 days after the onset of a spontaneous or progesterone induced menses and continued for 5 days (D3 – D7 of cycle) (31). US FDA recommends maximum dosage of 100 mg /day. Considerable clinical experience with CC indicates that a dosage up to 250 mg/d is safe (32).

If ovulation does not occur after 3 cycles of therapy, further treatment with CC is not recommended and the patient should be re evaluated.

ADVERSE EFFECTS	%
Ovarian enlargement	13.6%
Vasomotor flushes	10.4%
Abdominal pain / discomfort	5.5%
Distension / Bloating	
Nausea / vomiting	2.2%
Breast discomfort	2.1%
Visual symptoms	1.5%
(Blurred vision, lights, floaters, waves, photophobia, diplopic, scotomata)	
Headache	1.3%
AUB	1.3%

The most serious Complication of CC therapy-OHSS(ovarian Hyperstimulation syndrome)

CONTRAINDICATIONS

1. Hypersensitivity
2. Pregnancy
3. AUB of undetermined origin
4. Ovarian cyst
5. Uncontrolled adrenal or thyroid dysfunction or presence of intracranial lesion such as pituitary tumors.

CLOMIPHENE SET BACKS

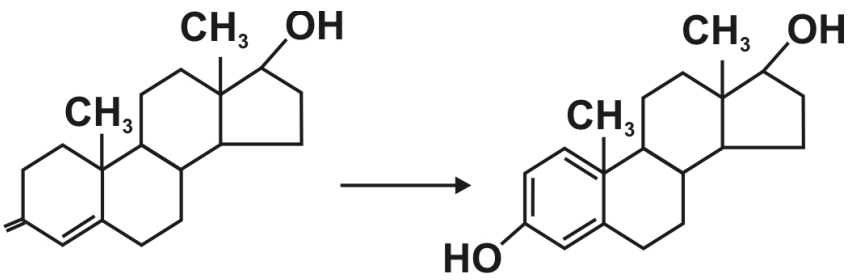
- Discrepancy between ovulatory and pregnancy rates
- Poor endometrium 30% (peripheral anti estrogenic effect)
- Thickness < 6 mm
- Decreased glandular density
- Decreased uterine blood flow
- Extended FSH window – Multifollicular ovulation
- Clomiphene resistance (20 – 25% fail to ovulate)

AROMATASE INHIBITORS

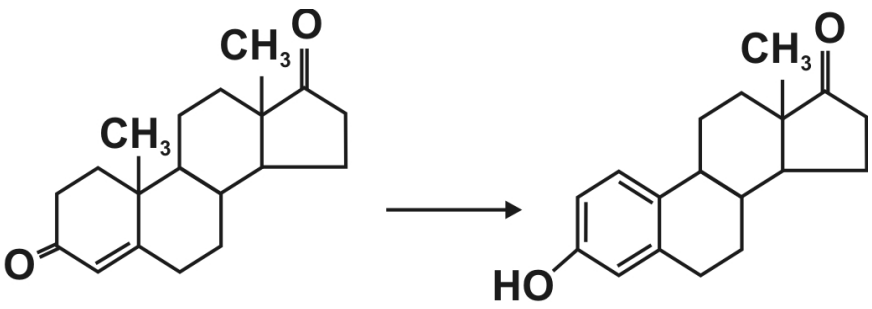
A large number of AIs have been developed over the last 30 yrs with the most recent, III generation AIs.

Aromatase is a microsomal cytochrome P450 hemoprotein containing enzyme (the product of CYP 19 gene) and catalyses the rate limiting step in the production of estrogen. Aromatase activity is also present in ovaries, brain, and adipose tissue, muscle, liver, breast tissue (33,34).

AROMATASE CONVERTS TESTOSTERONE TO ESTRADIOL



AROMATASE CONVERTS ANDROSTENEDIONE TO ESTRONE



CLASSIFICATION

Generation	Non steroidal	Steroidal
I	Aminoglutethimide	
II	Rogletimide, Fadrozole	Formestane
III	Anastrazole, Letrozole, Vorozole	Exemestane (suicide inhibitor)

Non selective - Aminoglutethimide, Testolactone (Teslac)

Selective - Anastrazole Zn 1033 (Arimidex)

Letrozole Gn 20267 (Femara)

Exemestane (Aromasin)

Vorozole (Rivizor)

Formestane (Lentaron)

Fadrozole (Afema)

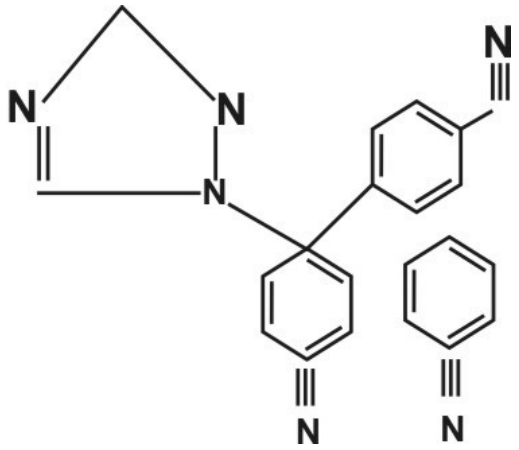
LETROZOLE

It is a third generation AI. Highly selective and highly potent inhibitor of Aromatase in vitro, in vivo in animals and in humans.

Molecular formula : $C_{17} H_{11} N_5$

Molecular weight : 285.30

MOLECULAR STRUCTURE (35) :



4-4'-(1H-1, 2, 4 - Triazol - 1 - yl methylene) diabenzonitrite ;

4-(C_4 - cyanophenyl) - (1,2,4 - Triazol - 1 - yl methyl)

MECHANISM OF ACTION

- Block estrogen negative feed back, without depletion of ER.
- Both circulating estrogen and locally produced estrogen in the brain exert negative feed back on gonadotropin release (38 – 41).
- Inhibition of aromatization → Block E production from all sources →

  Release HP axis from estrogenic negative feed → back increase in Gonadotropin secretion → growth of follicles (Casper and Mitwally) (42).
- Central feed back mechanisms remains intact. Suppression of FSH & atresia of smaller growing follicles occurs causing monoovulation.

IN PCOS

AI do not antagonize the ERs in the brain and the initiation of follicle growth accompanied by increasing concentrations of both estradiol and inhibin results in a normal negative feed back loop that limits FSH response, thereby avoiding the risk of high multiple ovulation and OHSS.

PERIPHERAL MECHANISM OF ACTION

- Increased follicular sensitivity to FSH.

- Temporary accumulation of intraovarian androgen – amplifies FSH effects – stimulates IGF–1 synergize with FSH to promote folliculogenesis (43-46).

CHANGES IN THE ENDOMETRIUM

- Upregulation of ERs in the endometrium leading to rapid endometrial growth.

- Increased endometrial sensitivity to estrogen resulting in more rapid proliferation of endometrial epithelium and stroma and improved flood flow to the uterus (49). So occurs normal endometrial development and thickness.

INDICATIONS

- Used alone for OI in WHO group II dysfunction.
- As an adjuvant in conjunction with exogenous FSH or other medications to improve the out come of OI.
- Induction of ovulation in CC failures (47).
- Along with FSH for super ovulation for IUI or IVF (50% reduction in dose of FSH required) (48).

DOSAGE

- Optimal dosage 2.5 – 5 mg from D3 – D7
- Single dose 10 – 30 mg on D3 shows similar rates of ovarian stimulation.
- Single high dose 60 mg has been reported without any negative effects.

PHARMACOKINETICS

- Letrozole is rapidly and completely absorbed (99.9% bioavailability).
- Extensively distributed to tissues.
- Large volume of distribution at steady state (1.87 lt/kg) range (1.47 – 3.24) and 60% bound to plasma proteins, mainly to albumin 55%.

- T½ of Letrozole – 42 hrs.

- Area under cure (AUC) is greater in patients with breast cancer due to reduced metabolic clearance. Metabolized to a pharmacologically inactive carbinol metabolite (4,4' – methanol bis benzo – nitrite) and excreted by kidneys. Of the radiolabel recovered in urine, 75% was the glucoronide of carbinol metabolite, 9% - 2 unidentified metabolites & 6% - unchanged letrozole.

In human liver microsomes letrozole strongly inhibited CYP2 A6 and moderately inhibited CYP2c 19.

POTENCY

I gen	II gen	IIIgen
Aminoglutethimide	➢ 4 – OHA 92% ➢ Fadrozole 93%	➢ Anastrazole 92% ➢ Vorozole 98% ➢ Letrozole 99%

DRUG INTERACTIONS

Co-administration of Letrozole and Tamoxifen 20 mg resulted in reduction of plasma levels of letrozole by 38% (36).

ADVERSE EFFECTS

❖ Hot flushes, nausea, vomiting, weight gain, tiredness, dizziness, Joint pain, unusual

sweating at night.

- ❖ Moderate decrease in lymphocyte counts
- ❖ Transient depression
- ❖ Thrombocytopenia
- ❖ Allergic reaction – rare.

Less common - Constipation

Vaginal dryness

Swelling

Troubled sleep

CONTRAINDICATIONS

In pregnancy

Embryologic and fetotoxic as indicated by

Intrauterine mortality

Increased resorption

Increased post implantation loss

Decreased number of live fetuses

Total anomalies including absence and

Shortening of renal papilla,

Dilation of ureter, edema and incomplete ossification of frontal skull and metatarsals

ADVANTAGES

- Mono follicular ovulation & decreased multiple pregnancy, OHSS & less monitoring
- Good Endometrium
- Convenience of administration & low cost
- Reduction in amount of FSH used for superovulation
- Good safety margin because of short half – life.

REVIEW OF LITERATURE

V ATAY, C CAM, M MUHCU et al 2006 compared Letrozole with CC as a first line treatment for OI in with PCOS. Randomized a total of 106 women with primary infertility. Concluded that Letrozole is associated with better ovulation induction 16.5% and higher pregnancy rate and as a first line treatment for anovulatory patients with PCOS.

A BILJAN AND COLLEAGUES (74) conducted prospective, randomized, controlled trial of CC Vs Letrozole for OI in women with unexplained infertility & concluded that Letrozole was an effective alternative to CC in view of monoovulation, endometrial thickness and blood flow. The pregnancy rate was 5.6% in the CC group and 10.7% in the Letrozole group.

BASIR et al (10) conducted a comparison study of Letrozole with CC in women with unexplained infertility and concluded that Letrozole is effective as a first line treatment for OI.

VENDOLA et al 1998 studied the use of Letrozole in OI with anovulatory infertility and concluded that high risk of ovarian hyperstimulation syndrome and multiple ovulation was less compared with CC.

GOSWARNI et al., **2004; SCHOOL CRAFT et al, 2004 ; VERPOEST et al., 2006** conducted clinical studies of AIs for ovarian stimulation in IVF and have shown that AIs could be a low – cost alternative to natural cycle IVF in patients who are poor responders to FSH

MITWALLY AND CASPER, 2001 conducted clinical studies of the use of Letrozole

for OI. Ovulation occured in 75% of patients and pregnancy was achieved in 25%

ELNASHAR et al., 2006 conducted studies of use of AIs for OI and concluded that Letrozole had an ovulation rate 54.6% and pregnancy rate 25% higher than CC.

ATAY et al, 2006 assessed the efficacy of AIs for OI compared with CC. 106 women with oligomenorrhea and PCOS were enrolled. Results were more favourable in the Letrozole group than in CC group regarding the percentage of ovulatory cycles (82.4% Vs 63.6%), pregnancy (21.6% Vs9.1%), monofollicular cycles (1.2 Vs 2.4 follicles > 18 mm on the day of hcG administration) and endometrial thickness (8.4 mm Vs 5.2 mm).

BAYER et al 2006, 36 patients (95 cycles) were given CC and 38 patients (95 cycles) given Letrozole concluded that Letrozole is better than CC in terms of ovulation rate (74.5% Vs 65.7%) or pregnancy rates (7.4% Vs 9%)

SOHRABVAND et al 2006 conducted studies on PCOS women resistant to CC and concluded that endometrial thickness was higher with Letrozole than CC.

BADAWY et al 2007 studied 438 infertile women with PCOS and concluded that ovulation rate (84% Vs 60%) was better in Letrozole group. Pregnancy rate (27 Vs 16.6%) and endometrial thickness (9.2 Vs 8.1 mm) was better in Letrozole group than CC group.

AIM OF STUDY

To study about the comparative efficacy of Letrozole Vs clomiphene citrate in ovulation Induction in patients with polycystic ovarian syndrome.

MATERIALS AND METHODS

STUDY DESIGN

Cohort study

A prospective, randomized, parallel, multicentric comparative trial

PERIOD OF STUDY

2008 – 2009

PLACE OF STUDY

Department of fertility research clinic, IOG, Egmore

SELECTION OF CASES

100 women belonging to eligible criteria of age (21 – 35 yrs) after obtaining informed consent from each participant.

	ARMS	ASSIGNED INTERVENTIONS
1.	Experimental	Drug: Letrozole 2.5 mg OD for 5 days (D3 – D7)
2.	Active Comparator	Drug: CC 50 mg OD for 5 days (D3 – D7)

INCLUSION CRITERIA

1. Age : 21 – 35 yrs

2. Period of Infertility

3. PCOS with at least 2 of Diagnostic criteria

   - Oligomenorrhea / Amenorrhea
   - Clinical evidence of Acne / hirsutism
   - USG evidence of PCOS

4. Normal semen analysis

5. Normal pelvic USG

6. Normal bilateral tubal patency

7. No recent treatment for OI (with in 6 months)

8. Willingness and giving written informed consent

EXCLUSION CRITERIA

1. Women with uterine /adnexal pathology (Eg) fibroid, ovarian cyst are excluded from the study.

2. Women with clinical features of hyperthyroidism, hypothyroidism are excluded.

3. Those with previous history of any surgeries related to genital tract as per history were

excluded.

4. Those with impaired hepatic / renal function were excluded

5. Women with RBS> 140 mg/d/ were excluded from the study

6. Age > 35 yrs and obese patients were excluded from this study

7. Lack of willingness.

The participants in this study were subjected to history, clinical and biochemical evaluation.

1. HISTORY

- **Age** (21 – 35 yrs) : An association between age of the woman and reduced fertility has been well documented (65). The decline in fecundability begins in early 30_s and accelerates during late 30_s and early 40_s. Oocyte related decline in fertility is due to decreased ovarian reserve (66).

- **Period of Infertility** : Failure to conceive during 12 – 18 mth despite unprotected regular coitus justifies full investigations. When the women age > 30 yrs and man > 40 yrs – investigations should not be delayed.

- **Menstrual History** : The patients included in the study were evaluated for history of

oligomenorrhea, amenorrhea & menorrhagia. In our study women with oligomenorrhea were subjected to the study after progesterone withdrawal bleed. Women with primary amenorrhea were excluded from our study.

- History related to previous surgeries related to genital tract, H/o appendicitis, peritonitis and H/o genital tract TB were excluded from this study.

CLINICAL EXAMINATION

The patients enrolled in our study were subjected to clinical examination which included.

1. All patients height and weight was measured. BMI was then calculated.

BMI = wt in kg. / Ht in m^2 It is a reliable indicator, Inexpensive and easy to perform.

Measurement units	Formula & Calculations
Kg & m (or cm)	wt (kg) / (Ht (m))^2
	Ht in cm by 100 = Ht in m
pounds & inches	wt (Ib) x (Ht (inches))^2 x 703
	conversation factor - 703

Interpretation

BMI	Weight status

< 18.5	Under weight
18.5 – 24.9	Normal
25.0 – 29.9	Over weight
30.0 – 39.9	Obese
40.0 & More	Extreme obesity

2. All patients waist (in cm) and WHR measured

Significance : Upper body obesity (male type) with a WHR > 0.9 associated with higher risk of long term morbidity (Pasquali et al., 1994).

3. Clinical evidence of acne, hirsutism, acanthosis nigricans, hyper thyroidism and hypothyroidism were looked for in all patients enrolled in our study.

Significance : Hirsutism and acne are the commonest manifestation and are associated with menstrual irregularities and obesity. Usually degree of hirsutism is quantified by Ferriman–Gallway scoring system. Score begins from o (no excusive terminal hair growth) to 4 (extensive terminal hair growth) & the numbers added up to maximum of 36.

USG evidence of PCOS : All patients had antral follicle count of at least 10 with size 2 – 9 mm. The ovarian volume = / > 10 ml. Evidence of fibroid & ovarian cyst / adnexal masses ruled out.

5. Semen Analysis WHO (2002) Normal Analysis:

Volume > 2ml

Sperm concentration	>	20 million / ml
Sperm motility	>	50% progressive or 25% rapidly progressive
Morphology	>	15% normal form
White blood cells	>	1 million / ml

Immuno bead or mixed anti globulin reaction test < 10% coated.

In our study, all had normal semen analysis.

III. Biochemical Investigations

1. Complete blood count, random blood sugar, renal function test and liver function test done in all patients. Any abnormality in any one of these investigations, those patients were excluded from this study.

2. For bilateral tubal patency – hysterosalpingography was performed. Those with abnormal tubal patency were excluded from our study.

RESULTS AND ANALYSIS

The selected randomized patients were allocated as Group L and Group CC, of which Group L patients were administered Letrozole 2.5 mg OD from D3-D7 of the menstrual cycle and Group CC patients were administered CC 50 mg OD from D_3-D_7 of the menstrual cycle. Both the group of patients, was subjected to transvaginal ultrasonographic monitoring of number of follicles, Maximum diameter of the largest follicle, and endometrial thickness from D_{11} onwards. The serum progesterone levels were measured on Day 21 which indicates the definite occurrence of ovulation. The treatment was discontinued in both the groups, if patients, developed ovarian enlargement.

The number of follicles, mean diameter of the largest follicle, ovulation rate, endometrial thickness and serum progesterone values were compared in the two groups. A group test or the students test was used to compare data as appropriate. A value < 0.05 was considered to be statistically significant.

TABLE - 1

AGE GROUP - CROSS TABULATION

Age Group		Group		Total
		L	CC	
21-25 Yrs.	Count	72	71	143
	% within Group	72.0%	71.0%	71.5%
26-30 Yrs.	Count	20	18	38
	% within Group	20.0%	18.0%	19.0%
31-35 Yrs.	Count	8	11	19
	% within Group	8.0%	11.0%	9.5%
Total	Count	100	100	200
	% within Group	100.0%	100.0%	100.0%

CHI-SQUARE TESTS

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-square	0.586[a]	2	0.746
Likelihood Ratio	0.588	2	0.745
Linear-by-Linear Association	0.187	1	0.665
N of Valid Cases	200		

a 0 cells (.0%) have expected count less than 5. The minimum expected count is 9.50.

GROUP STATISTICS

Age Group	N	Mean	Std. Deviation	Std. Error Mean
L	100	24.8800	3.18862	0.31886
CC	100	25.1600	3.32338	0.33234

The table shows the mean, standard deviation, in both groups L & CC with respect to age the mean age in Group L is 24.8 years. The mean age in Group CC is 25.1 years. Both groups are comparable, by applying the chi-square test, chi-square value 0.586 and p-value 0.746 are obtained. P value being > 0.05 is not significant.

TABLE - 2

MEAN DURATION OF INFERTILITY

Independent Samples Test

Group Statistics

Duration of Infertility	Group	N		Std. Deviation	p Value
	L	100	2.4400	0.60836	0.906
	CC	100	2.4300	0.59041	

The mean duration of infertility in Group L is 2.44 years and in Group CC is 2.43 years with p value is 0.906 (> 0.05). Not significant Both the groups are comparable with respect to mean duration of infertility.

TABLE - 3

GROUP STATISTICS

BMI	Group	N		Std. Deviation	p Value
	L	100	22.5300	3.13831	0.747
	CC	100	22.6880	3.74226	

The mean body mass Index in Group L is 22.53 with S.D. of 3.138. The mean body mass Index in Group CC is 22.68 with S.D. of 3.742.

Both the groups are comparable with respect to body mass Index with p value 0.747 (> 0.05) not Significant.

TABLE - 4

GROUP STATISTICS

Wait Hip Ratio	Group	N		Std. Deviation	p Value
	L	100	0.8276	0.2675	0.939
	CC	100	0.8273	0.2831	

The mean WHR in Group L is 0.8276 with S.D. of 0.267 Group CC is 0.827 with S.D. of 0.283.

Both the groups are comparable with respect to WHR with p value 0.939 (>0.05) Not Significant.

TABLE - 5

HIRSUTISM GROUP

Hirsutism		Group		Total
		L	CC	
No Hirsutism	Count % within Group	83 83.0%	78 78.0%	161 80.5%
Hirsutism	Count % within Group	17 17.0%	22 22.0%	39 19.5%
Total	Count % within Group	100 100.0%	100 100.0%	200 100.0%

CROSS TAB

Oligo Menorrhea		Group		Total
		L	CC	
No Oligo Menorrhea	Count % within Group	74 74.0%	73 73.0%	147 73.5%
Oligo Menorrhea	Count % within Group	26 26.0%	27 27.0%	53 26.5%
Total	Count % within Group	100 100.0%	100 100.0%	200 100.0%

AMENORRHEA GROUP

CROSS TAB

Amenorrhea		Group		Total
		L	CC	
No Amenorrhea	Count % within Group	83 83.0%	77 77.0%	160 80.0%
Amenorrhea	Count % within Group	17 17.0%	23 23.0%	40 20.0%
Total	Count % within Group	100 100.0%	100 100.0%	200 100.0%

17% of the cases in Group L had clinical evidence of hirsutism. 22% of the cases in Group CC had hirsutism.

26% of the cases in Group L and 27% of the cases in Group CC had oligomenorrhea.

17% of the cases in Group L and 23% of the cases in Group CC has history of amenorrhea.

After excluding pregnancy by urine gravindex method, these patients were subjected to the study after progesterone withdrawal bleed.

TABLE - 6

OVULATION GROUP

Ovulation		Group		Total
		L	CC	
Not Ovulated	Count % within Group	33 33.0 %	48 48.0%	81 40.5%
Ovulated	Count % within Group	67 67.0 %	52 52.0 %	119 59.5 %
Total	Count % within Group	100 100.0%	100 100.0%	200 100.0%

CHI-SQUARE TESTS

		df	Asymp. Sig. (2 sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-square	9.778[b]	1	0.002		
Continuity Correction	8.909	1	0.003		
Likelihood Ratio	9.862	1	0.002		
Fisher's Exact Test				0.003	0.001
Linear-by-Linear					
Association	9.729	1	0.002		
N of Valid Cases	200				

The ovulation response group tabulation shows that of 100 patients in L group, 67% ovulation occurred. 52% ovulation rate occurred in CC group. By applying chi-square tests, chi-square value 8.909 p value 0.003. (<0.05) = Significant. This shows that there is statistically significant difference in ovulation induction rate between the group of patients treated patients with L and CC. Letrozole treated patients had better ovulation rates than CC.

NUMBER OF FOLLICLES IN L & CC GROUP

Number of follicles		Group		Total
		L	CC	

No Follicle	Count % within Ovulation	33 33.0%	48 48.0%	81 40.5%
One Follicle	Count % within Ovulation	52 77.6%	6 11.5%	58 29.0%
Two Follicles	Count % within Ovulation	15 22.4%	23 44.2%	38 19.0%
Three Follicles	Count % within Ovulation	0 0.0%	23 44.2%	23 11.5%
Total	Count % within Ovulation	67 100.0%	52 100.0%	200 100.0%

CHI-SQUARE TESTS

		df	**Asymp. Sig. (2 sided)**
Pearson Chi-square	100.000[a]	3	0.000
Likelihood Ratio	138.469	3	0.000
Linear-by-Linear Association	84.366	1	0.000
N of Valid Cases	100		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is 2.88.

The cross tabulation shows that of 100 patients studied in Letrozole group, 52% of the cases developed single follicle, 15% of the cases developed two follicles. 33% of the cases did not ovulate with Letrozole.

Of 100 patients in CC group, 6% of the cases developed single follicle, 23% of the cases developed two follicle, 23% of the cases developed three follicles. 48% of the cases did not ovulate with CC therapy. By applying the chi-square tests, chi-square value 63.945 and p value

= .000 (< 0.05) highly significant.

Letrozole treated cases had better monofollicular ovulation rates than CC treated group.

TABLE 8

GROUP STATISTICS

Size of Follicle	Group	N		Std. Deviation	p Value
	L	67	21.1560	2.40499	< 0.05
	CC	52	18.7635	2.35614	

The table shows the mean diameter of largest follicle in L group is 21.15 mm with a S.D. of 2.404. In CC group, the mean diameter is 18.76 mm with a S.D. of 2.356 p value of 0.000 (<0.05) obtained which is highly significant.

TABLE 9

GROUP STATISTICS

Endometrial Thickness	Group	N		Std. Deviation	p Value
	L	67	8.6090	0.43753	0.000 (<0.05)
	CC	52	7.1115	0.60929	

The table shows the mean endometrial thickness in L group is 8.60 mm with a S.D. of 0.437.

In CC group the mean Endometrial thickness is 7.11 m with a S.D. of 0.609 P value obtained is 0.000 (<0.05) = High Significant.

TABLE – 10

GROUP STATISTICS

Serum Progesterone Level	Group	N		Std. Deviation	Std. Error Mean
	L	67	13.4164	1.57688	0.000
	CC	52	12.2788	1.87191	(< 0.05)

The table shows the mean serum progesterone level in L group is 13.41 ng/ml CC group is 12.27 ng/ml. p value of 0.000 (< 0.05) = Highly Significant.

DISCUSSION

A prospective randomized study was conducted in IOG during January 2008- Feb 2009. Of 200 patients who attended the infertility clinic 100 patients recruited in L group were allocated to receive 2.5 mg OD from D_3-D_7 of the menstrual cycle. 100 patients recruited in CC group were allocated to receive 50 mg OD from D_3-D_7 of the menstrual cycle. Both groups were comparable with respect of age, mean duration of infertility, BMI and WHR which had no statistically significant difference.

The patients recruited in both the groups were evaluated for evidence of acne, hirsutism, oligo / amenorrhea.

Our patients enrolled in both the groups were also evaluated for the presence of clinical evidence of hyperthyroidism and hypothyroidism.

In our study 17% of cases in L group and 22% of cases in CC group had hirsutism. 17% of cases in L group and 23% of cases in CC group had a history of amenorrhea. After excluding pregnancy for these patients by performing an urine gravindex method, these patients were included in our study after progesterone withdrawal bleed.

Both group were compared with respect of number of follicles, mean diameter of largest follicle, mean endometrial thickness and serum progesterone levels. The patients enrolled in both groups had no complications pertaining to L and CC during our period of study.

BADAWY *et al.,* 2007 studied 438 infertile women with polycystic ovarian syndrome and concluded that ovulation rates was better in L group (84% Vs 60%) and endometrial thickness was better in L group than CC group (9.2 vs 8.1 mm).

In our study, the ovulation rate in L group was 67% and that of CC group 52%

VATAY, C CAM M MUHCU *et al.,* in his study concluded that L is associated with better ovulation rate and can be used as first line treatment for anovulatory patients with polycystic ovarian syndrome.

In our study, 52% of the cases had monofollicular ovulation in L group where as in CC group only 6% of the cases developed single follicle and 23% of the cases developed two follicles.

BILJAN AND COLLEAGUES in his study concluded that Letrozole was an effective alternative to CC in view of monoovulation.

BASIR *et al*., in his study concluded that L is an effective alternative to CC for OI.

In our study, the mean endometrial thickness was better in Letrozole group than CC group (8.6 mm vs 7.1 mm).

The mean endometrial thickness (7.1 mm) in CC group indicates the adverse effects of CC on the endometrial growth that is thought to be due to depletion of the endometrial receptors.

SOHRABVAND *et al.,* in his study concluded that Endometrial thickness was higher

with L group than CC Group.

In our study the mean diameter of largest follicle was greater in L group than CC group (21.15mm Vs 18.76 mm). The serum progesterone levels measured on D21 indicated the definite occurrence of ovulation was 13.41 ng/ml in L group and 12.27 ng/ml in CC group.

MITWALLY *et al.,* in his study reported that use of letrozole is associated with significantly lower multiple pregnancy rates they explained this results by the fact that Letrozole induce limited number of mature follicles.

In a similar study, sh Tehrani Nejad, *et al.,* (Journal of Assisted Reprod and Genet, 2008) demonstrated that Letrozole was a better alternative to cc-gonadotropin in patients with unexplained infertility (63).

BEGUM MR, FERDOUS J, QUADIR E in his studies concluded that Letrozole has better ovulation and pregnancy rates in comparison to CC in patients with PCOS (64).

SUMMARY

In the patients recruited in this study,

1. The mean age in L group was 24.8 yrs and in CC group 25.1. years.

2. The mean duration of infertility in L group was 2.43 years and in CC group 2.44 years.

3. In Letrozole group, the ovulation rate was 67% and in CC group 52%.

4. In Letrozole group, 52% of the cases developed single dominant follicle and 15% of the cases had developed two dominant follicles.

5. In CC group only, 6% of the cases had monofollicular ovulation and 23% of the cases developed two follicles.

6. The mean diameter of largest follicle in L group is 21.15 mm and in CC group 18.76 mm.

7. The mean endometrial thickness is L group is 8.6 mm and in CC group 7.1 mm.

8. The serum progesterone level in L group is 13.41 ng/ml and CC group is 12.27 ng/ml.

CONCLUSION

Letrozole is more effective in inducing ovulation in patients with an ovulatory cycles than clomiphene citrate in terms of monofollicular ovulation and a better endometrial thickness than when compared with clomiphene citrate. The mean diameter of the largest follicle and the serum progesterone level studied showed letrozole was superior than clomiphene citrate in inducing ovulation. Hence Letrozole can be recommended as the first line drug for ovulation induction in anovulatory patients with polycystic ovarian syndrome.

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PROFORMA

NAME :

AGE :

OCCUPATION :

SOCIO ECONOMIC STATUS :

MARITAL STATUS :

PERIOD OF INFERTILITY :

HEIGHT :

WEIGHT :

BODY MASS INDEX ($kglm^2$) :

WAIST (CM) :

WAIST HIP RATIO :

SEMEN ANALYSIS :

MENSTRUAL HISTORY :

OLIGOMENORRHEA :

AMENORRHEA :

CLINICAL EVIDENCE OF :

ACNE :

HIRUSTISM :

ACANTHOSIS NIRGICANS :

HYPERTHYROIDISM :

HYPOTHYROIDISM :

USG EVIDENCE OF PCOS :

INVESTIGATIONS

COMPLETE BLOOD COUNT :

RENAL FUNCTION TEST :

LIVER FUNCTION TEST :

USG – FIBROID, OVARIAN CYST :

HYSTEROSALPINGOGRAM – FOR TUBAL PATENCY

PREVIOUS HISTORY OF SURGERY – YES / NO

RECENT TREATMENT FOR OVULATION INDUCTION
(WITH IN 6 MONTHS) – YES / NO

ABBREVIATIONS

FSH	-	Follicle stimulating hormone
LH	-	Leutinizing harmone
CAMP	-	Cyclic Adenosine monophosphate
LDL	-	Low density Lipoprotein
HCG	-	Human chorionic Gonadotrophin
FP	-	Follicular Phase
L.P.	-	Luteal Phase
POD	-	Pouch of Douglas
PCOS	-	Polycystic ovarian syndrome
CAH	-	Congenital Adrenal Hyperplasia
MRKA	-	Meyer – Rokitansky Kuster Hauser syndrome
SOL	-	Space occupying lesion
PrL	-	Prolactin
NICHD	-	National Institute for Child Health and Human Development
ESHRE	-	European Society for Human Reproduction
ASRM	-	American Society for Reproductive Medicine
AES	-	Androgen Excess Society
TGL	-	Triglycerides
HDL	-	High Density Lipoprotein
BMI	-	Body mass Index
WHR	-	Waist Hip Ratio
PAI	-	Plasminogen Activator Inhibitor
OI	-	Ovulation Induction
OHSS	-	Ovarian Hyperstimulation Syndrome
HSG	-	Hysterosalpingrography
SERMs	-	Selective Oestrogen Receptor Modulators
GnRH	-	Gonadotropin Releasing Harmone
HMG	-	Human Menopausal Gonadotropin
OCP	-	Oral contraceptive pill
L	-	Letrozole
CC	-	Clomiphene Citrate
HRT	-	Hormone Replacement Therapy
TVS	-	Transvaginal Ultrasonography
AUB	-	Abnormal Uterine Bleeding
AI	-	Aromatase Inhibitors
ER	-	Estrogen Receptor
HPA	-	Hypothalamic Pituitary Axis
IGF-1	-	Insulin like Growth factor – 1
RBS	-	Random Blood Sugar

KEY TO MASTER CHART

Sl.No.	-	Serial Number
M/s.	-	Duration of Infertility
Ht	-	Height
Wt	-	Weight
BMI	-	Body Mass Index
WHR	-	Waist Hip Ratio
Oligo	-	Oligomenorrhea
Amen	-	Amenorrhea
EMT	-	Endometrial Thickness
Prog	-	Progesterone
Group 1	-	Letrozole
Group 2	-	Clomiphene citrate